

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

86/42/80  
**F & BERRIDGE, PLC**  
 Box 19928  
 Alexandria, Virginia 22320  
 Phone: (703) 836-6400  
 Facsimile: (703) 836-2787

Attorney Docket No.: WPB 36400B

Date: August 24, 1998

jc588 U.S. PRO  
 09/138735  
 08/24/98

Assistant Commissioner for Patents  
 Washington, D.C. 20231

BOX PATENT APPLICATION  
CONTINUING APPLICATION TRANSMITTAL  
RULE 1.53(b)

Sir:

Transmitted herewith for filing under 37 C.F.R. §1.53(b) is a

Continuation       Divisional       Continuation-in-Part

application of prior pending Application No. 08/480,917, filed June 7, 1995,

For (Title): TRYPANOSOMA CRUZI ANTIGEN, GENE ENCODING THEREFOR AND  
 METHODS OF DETECTING AND TREATING CHAGAS DISEASE

By (Inventors): Glaucia PARANHOS-BACCALA; Mylene LESENECHAL and Michel JOLIVET

1.  A Declaration and Power of Attorney is attached. The attached Declaration and Power of Attorney is:
  - a. A copy of the Declaration and Power of Attorney from the parent application. (Used with the same or fewer inventors and (a) a copy of the prior application or (b) a revised, reformatted or edited version of the prior application that does not contain new matter.)
  - b. A new Declaration and Power of Attorney. (Used with the same, fewer or additional inventors and (a) a copy of the prior application, (b) a revised, reformatte or edited version of the prior application that does not contain new matter, or (c) a new specification.)
2.  The filing fee is calculated below:

CLAIMS IN THE APPLICATION AFTER ENTRY OF  
 ANY PRELIMINARY AMENDMENT NOTED BELOW

FOR:	NO. FILED	NO. EXTRA
BASIC FEE		
TOTAL CLAIMS	20 - 20	= 0*
INDEP CLAIMS	1 - 3	= 0*
<input type="checkbox"/> MULTIPLE DEPENDENT CLAIMS PRESENTED		

\* If the difference is less than zero, enter "0".

SMALL ENTITY

RATE	FEES
	\$ 395
x 11 =	\$
x 41 =	\$
+135 =	\$
TOTAL	\$

OTHER THAN A  
 SMALL ENTITY

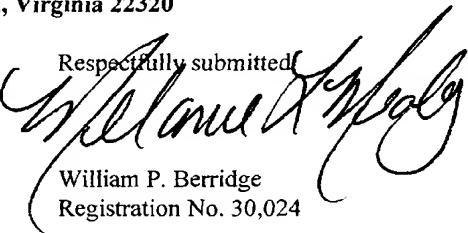
OR	RATE	FEES
OR		\$ 790
OR	x 22	\$ -----
OR	x 82	\$ -----
OR	+270	\$ -----
OR	TOTAL	\$ 790

3.  Check No. 61059 in the amount of \$790.00 to cover the filing fee is attached. The Commissioner is hereby authorized to charge any other fees that may be required to complete this filing, or to credit any overpayment, to Deposit Account No. 15-0461. Two duplicate copies of this sheet are attached.
4.  Cancel claims \_\_\_\_\_ of the application before calculating the filing fee. At least one independent claim is retained for filing purposes.

DEPOSIT ACCOUNT USE AUTHORIZATION Please grant any extension necessary for entry; Charge any fee due to our Deposit Account No. 15-0461
--

5.  Amend the specification by inserting before the first line the sentence:  
--This is a  Continuation  Division  Continuation-in-Part of Application No. 08/480,917 filed June 7, 1995. The entire disclosure of the prior application(s) is hereby incorporated by reference herein in its entirety.--
6.  Formal drawings (Figs. 1-2) are attached.
7.  Priority of foreign application No. 94 10132 filed August 12, 1994 in France is claimed under 35 U.S.C. §119 and/or §365(b).  
 The certified copy was filed in prior Application No. 08/480,917 on September 7, 1995.  
 A certified copy of the above foreign application(s) is filed herewith.
8.  Priority of U.S. Provisional Application(s) No. \_\_\_\_\_ filed \_\_\_\_\_ is claimed under 35 U.S.C. §119.  
 Amend the specification by inserting before the first line the sentence:  
--This nonprovisional application claims the benefit of U.S. Provisional Application(s) No. \_\_\_\_\_ filed \_\_\_\_\_.
9.  The prior application is assigned of record to BIO MERIEUX recorded at Reel 7626, Frame 0182.
10.  This application is filed by fewer than all the inventors named in the prior application (37 C.F.R. §1.53(d)(4)). Delete the following inventor(s) named in the prior application:  
\_\_\_\_\_  
\_\_\_\_\_
11.  A Preliminary Amendment is attached. Claims added by this Amendment are properly numbered consecutively beginning with the number next following the highest numbered claim in the application.
12.  An Information Disclosure Statement is attached.
13.  Small entity status:  
 a. A small entity statement is attached.  
 b. A small entity statement was filed in the parent application and such status is still proper and desired.  
 c. Small entity status is no longer claimed.
14.  Other: \_\_\_\_\_
15.  The power of attorney in the application is to James A. Oliff, Registration No. 27,075, William P. Berridge, Registration No. 30,024, Kirk M. Hudson, Registration No. 27,562, Thomas J. Pardini, Registration No. 30,411, Edward P. Walker, Registration No. 31,450, Robert A. Miller, Registration No. 32,771 and/or Mario A. Costantino, Registration No. 33,565.  
 a. The power appears in the attached Declaration and Power of Attorney.  
 b. Since the power does not appear in the attached Declaration and Power of Attorney, a substitute Power of Attorney is also attached.
16.  Address all future communications to:

OLIFF & BERRIDGE, PLC  
P.O. Box 19928  
Alexandria, Virginia 22320

Respectfully submitted,  
  
William P. Berridge  
Registration No. 30,024

Melanie L. Mealy  
Registration No. 40,085

**PATENT APPLICATION**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of

Glaucia PARANHOS-BACCALA; Mylene  
LESENECHAL and Michel JOLIVET

Prior Group Art Unit: 1641

Application No.: Rule 1.53(b) Div. Appln. of U.S.  
Appln. No. 08/480,917, filed June 7, 1995

Prior Examiner: J. Shaver

Filed: August 24, 1998

Docket No.: WPB 36400B

For: TRYpanosoma CRUZI ANTIGEN, GENE ENCODING THEREFOR AND  
METHODS OF DETECTING AND TREATING CHAGAS DISEASE

**PRELIMINARY AMENDMENT**

Assistant Commissioner of Patents  
Washington, D. C. 20231

Sir:

Prior to initial examination, please amend the above-identified application as follows:

**IN THE TITLE:**

Page 1, above the first line, insert the following title: --TRYpanosoma CRUZI  
ANTIGEN, GENE ENCODING THEREFOR AND METHODS OF DETECTING AND  
TREATING CHAGAS DISEASE--

**IN THE SPECIFICATION:**

Please amend the specification as follows:

Page 1, before line 1, insert:

--FIELD OF THE INVENTION--;

between lines 5 and 6, insert:

## --BACKGROUND OF THE INVENTION--

- Page 2, line 1, change "epi-mastigote" to --epimastigote--;  
line 26, change "tests, such as" to --tests include--.

Page 3, line 29, change "where" to --were--.

Page 4, line 1, change "sero-logical" to --serological--;  
line 4, change "anti-gen" to --antigen--;  
between lines 6 and 7, insert:  
--SUMMARY OF THE INVENTION--  
between lines 22 and 23, insert:  
--DETAILED DESCRIPTION OF THE INVENTION--

line 26, change "No.1," to --NO:1--;  
line 28, change "No.1," to --NO:1--.

Page 5, line 5, change "No.1" to --NO:1--.

Page 7, line 10, change "No.1" to --NO:1--;  
line 12, change "No.1" to --NO:1, and--;  
line 13, delete "and" (first occurrence);  
line 14, after "2207" insert --of SEQ ID NO:1--;

line 26, change "No.2." to --NO:2--.

Page 8, line 3, change "No.2," to --NO:2--.

Page 9, line 4, change "(E" to --(E--.

Page 10, line 7, change "No.2." to --NO:2--.

Page 13, line 30, change "under-stood" to --understood--.

Page 15, line 19, change "No.1," to --NO:1,--;

line 24, change "No.1," to --NO:1,--.

Page 16, line 6, change "No.1." to --NO:1,--;

line 29, change "techniques," to --technique,--;

line 30, after "(14)),," insert --the--;

line 32, before "Northern" insert --the--.

Page 17, line 2, after "target," insert --and the--;

line 4, change "tech-nique" to --technique--;

line 13, change "trans-lation" to --translation--;

line 14, change "pheno-mena." to --phenomena.--;

line 24, change "No.7," to --NO:7,--; change "No.8," to --NO:8,--; change  
"No.9," to --NO:9,--; change "No.10," to --NO:10, and--;

line 25, change "No.12." to --NO:12.--.

Page 18, between lines 12 and 13, insert:

**--DESCRIPTION OF THE FIGURES--**

Page 19, line 8, change "No.3" to --NO:3--; change "24," to --24--;

line 10, change "No.4" to --NO:4--; change "24" to --24,--;

line 25, change "No.I" to --NO:1--;

line 26, change "No.2," to --NO:2,--;

line 28, change "1825." to --1825 of SEQ ID NO:1,--;

line 30, change "No.2." to --NO:2,--.

Page 20, line 23, change "For that, microtiter" to --Microtiter--;

line 24, change "name), nunc)" to --name))--;

line 30, delete "entire";

line 31, change "react" to --reacts--.

Page 21, line 31, change "Tc 50" to --Tc50--.

Page 22, line 17, change "No.5" to --NO:5--;

line 18, change "No.6" to --NO:6--;

line 20, change "No.5" to --NO:5--;

line 22, change "No.6" to --NO:6--;

line 26, change "No.1," to --NO:1,--.

Page 23, line 18, change "No.7" to --NO:7--;

line 19, change "No.8" to --NO:8--;

line 21, change "No.7" to --NO:7--;

line 25, change "No.8" to --NO:8--;

line 28, change "No.1," to --NO:1,--.

Page 24, line 3, change "No.1" to --NO:1--;

line 9, change "No.9 :" to --NO:9:--;

line 11, change "No.10:" to --NO:10:--;

line 13, change "No.9" to --NO:9--;

line 16, change "No.1." to --NO:1,--;

line 17, change "No.10" to --NO:10--;

line 20, change "No.10," to --NO:10--;

line 25, change "No.1" to --NO:1--.

Page 25, line 1, change "No.11" to --NO:11--;  
line 7, change "No.12:" to --NO:12:--;  
line 9, change "No.13:" to --NO:13:--;  
line 11, change "No.12" to --NO:12--;  
line 15, change "No.11" to --NO:11--;  
line 17, change "No.11." to --NO:11.--;  
line 19, change "des-cribed," to --described,--;  
line 22, change "No.1" to --NO:1--.

Page 37, line 23, change "1426" to --426-428--.

Page 38, line 21, change "386-389" to --836-839--.

REMARKS

By this Preliminary Amendment, the specification has been amended to place the application in better condition for initial examination and allowance. Claims 1-20 are pending.

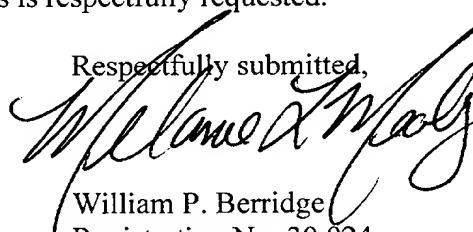
The computer readable form of the Sequence Listing in this application is identical with that filed in Application No. 08/480,917, filed on June 7, 1995. In accordance with 37 C.F.R. §1.821(e), please use the computer readable form filed in that application as the computer readable form for the instant application. It is understood that the U.S. Patent and Trademark Office will make the necessary change in application number and filing date for the computer readable form that will be used for the instant application.

Amino acids 321-340 were inadvertently omitted from SEQ ID NO:2 on page 28 of the French-language patent application, filed June 7, 1995, and from SEQ ID NO:2 on page 32 of the Verified Translation. The amino acid sequence identified in SEQ ID NO:2

corresponds to the nucleotide sequence identified in SEQ ID NO:1. One of ordinary skill in the art would have recognized from these disclosures that a segment of amino acids was missing from SEQ ID NO:2 underneath identification numbers 321-340 and would have readily been able to determine the omitted amino acid segment by translating from the corresponding codons in SEQ ID NO:1. Thus, no new matter is added.

Early and favorable action on the merits is respectfully requested.

Respectfully submitted,

  
William P. Berridge  
Registration No. 30,024

Melanie L. Mealy  
Registration No. 40,085

WPB:CLC/jca

OLIFF & BERRIDGE, PLC  
P.O. Box 19928  
Alexandria, Virginia 22320  
Telephone: (703) 836-6400

The subject of the present invention is a new genetic material encoding a new protein recognized by anti-Trypanosoma cruzi antisera, and it relates to the use of said gene and protein, especially for diagnostic, pharmaceutical and therapeutic purposes.

Trypanosoma cruzi is a flagellate protozoal parasite, a member of the order Kinetoplastida and of the family Trypanosomatidae, which is responsible for Chagas disease which affects naturally millions of persons, mainly in Latin America.

In vertebrate hosts, Trypanosoma cruzi is present in two forms: one which is mobile by means of its flagellum or trypomastigote and which does not divide; the other is aflagellate, or intracellular amastigote, which multiplies by binary division.

Transmission of the protozoan in man occurs through hematophagous insects of the family Reduviidae, during a blood meal followed by dejections at the site of the bite. The vector insect thus releases the infectious metacyclic trypomastigote forms which will colonize many cell types through the blood circulation. Trypanosoma cruzi infects cardiac and skeletal muscular cells, the glial cells and the cells of the mononuclear phagocytic system. After passive penetration into the host cell, the trypomastigote form of the parasite differentiates into the amastigote form, divides actively and then this is followed by a release of the trypomastigote forms, thereby causing a new cell invasion.

The insects will complete the parasitic cycle by ingesting, during a blood meal, the trypomastigote forms in the host. The latter differentiate into

epi-mastigote forms in the vector's middle intestine and finally into the infectious metacyclic trypomastigote forms in the posterior intestine.

Two phases can be distinguished in the Chagas disease: the acute phase and the chronic phase. The acute phase occurs after a transfusional, congenital or vectorial type contamination and lasts for a few weeks. It is characterized by a large number of parasites circulating in the blood and corresponds to an exponential division of the protozoan. The acute phase is most often asymptomatic. However, in infants contaminated by their mother, the acute phase, which is marked by an acute cardiopathy, may be critical. The chronic phase may extend over many years. In some individuals, this phase is asymptomatic. On the other hand, other patients have tissue lesions in the heart or digestive type manifestations. In any case, clinical diagnosis must always be confirmed by tests for the detection either of antibodies directed against the parasitic antigens, or of the parasite itself.

This disease is becoming a worldwide problem because of the contamination through blood transfusion. It was therefore becoming essential to have available diagnostic tests which make it possible to determine the presence of the parasite in individuals. Various serological tests, such as direct agglutination, indirect immunofluorescence (IIF), complement fixation tests (CFR), ELISA tests (Enzyme Linked Immunosorbent Assay). The *Trypanosoma cruzi* antigens used for the serological tests are obtained from a total lysate of the noninfectious stage of the parasite or from partially purified protein fractions. However, these

fractions do not allow antigens to be obtained in sufficient quantity and quality for the production of a reliable serological diagnostic test. Furthermore, the complexity of the parasite and the strain-to-strain 5 antigenic polymorphism introduce an additional difficulty in the reproducibility of the different preparations. Finally, there are many risks of cross-reactivity with other protozoa, more particularly with *Trypanosoma rangeli*, a nonpathogenic parasite, and the 10 family *Leishmania*. Another disadvantage of these techniques is the absence of determination of the disease phase which would allow a treatment from the onset of the acute phase.

In order to solve these various problems, it 15 was envisaged to produce a serological diagnostic kit composed of recombinant proteins which would be specific for *Trypanosoma cruzi*.

Various research groups have screened libraries for expression of *Trypanosoma cruzi* genomic DNA or 20 complementary DNA in the vector  $\lambda$ gt11, using sera from patients suffering from Chagas disease. The  $\lambda$ gt11 phage allows the insertion of foreign DNA of a maximum size of 7Kb into the EcoR1 site localized in the lacZ gene, under the control of the lac promoter. The product 25 obtained is a recombinant protein used with beta-galactosidase, which is inducible by IPTG (isopropyl beta-D-thiogalactoside).

Various *Trypanosoma cruzi* genes, encoding proteins recognized by the Chagasic sera were thus 30 characterized. Among the recombinant antigens described, the H49 antigen may be mentioned (Paranhos et al., 1994 (1)). However, this antigen does not allow a

sero-logical detection sensitivity of 100% of the patients in the acute or chronic phase. It was therefore envisaged to combine the H49 antigen with the CRA anti-gen (Cytoplasmic Repetitive Antigen) (Lafaille et al., (1989) (2)) but still without solving this problem.

The present inventors have identified and obtained for the first time a new genetic material encoding a new protein, recognized by anti-Trypanosoma cruzi antisera, which makes it possible to overcome the abovementioned disadvantages. The genetic material may be used to produce proteins or polypeptides for the production of diagnostic tests, or for the preparation of vaccinal or pharmaceutical compositions, or may 15 itself either be used as a probe, or for the determination of specific probes which can be used in nucleic acid hybridization tests for the detection of Trypanosoma cruzi infections. Likewise, the protein or any corresponding polypeptide may be used for the production of antibodies specific for the parasite, for 20 diagnostic or passive protection purposes.

This gene was called Tc 100 by the applicant.

Consequently, the subject of the present invention is a DNA or RNA molecule consisting of at least one strand comprising a nucleotide sequence represented in the identifier SEQ ID No.1, or a sequence complementary or antisense or equivalent to said sequence identified in the identifier SEQ ID No.1, and especially a sequence having, for any succession of 25 100 contiguous monomers, at least 50%, preferably at least 60%, or better still at least 85% homology with 30 said sequence.

Nucleotide sequence is understood to mean either a DNA strand or its complementary strand, or an RNA strand or its antisense strand or their corresponding complementary DNAs. The DNA sequence as 5 represented in the identifier SEQ ID No.1 corresponds to the messenger RNA sequence, it being understood that the thymine (T) in the DNA is replaced by a uracil (U) in the RNA.

According to the invention, two nucleotide 10 sequences are said to be equivalent in relation to each other, or in relation to a reference sequence if, functionally, the corresponding biopolymers can play essentially the same role, without being identical, with respect to the application or use considered, or in the 15 technique in which they are involved; two sequences obtained because of the natural variability, especially spontaneous mutation, of the species from which they were identified, or because of induced variability, as well as homologous sequences, homology being defined 20 below, are especially equivalent.

Variability is understood to mean any spontaneous or induced modification of a sequence, especially by substitution and/or insertion and/or deletion of nucleotides and/or of nucleotide fragments, 25 and/or extension and/or shortening of the sequence at at least one of the ends; a nonnatural variability may result from the genetic engineering techniques used; this variability may result in modifications of any starting sequence, considered as reference, and capable 30 of being expressed by a degree of homology relative to the said reference sequence.

Homology characterizes the degree of identity of two nucleotide (or peptide) fragments compared; it is measured by the percentage identity which is especially determined by direct comparison of nucleotide (or peptide) sequences, relative to reference nucleotide (or peptide) sequences.

Any nucleotide fragment is said to be equivalent to a reference fragment if it has a nucleotide sequence which is equivalent to the reference sequence; according to the preceding definition, the following are especially equivalent to a reference nucleotide fragment:

- a) any fragment capable of at least partially hybridizing with the complementary strand of the reference fragment,
- b) any fragment whose alignment with the reference fragment leads to the detection of identical contiguous bases, in greater number than with any other fragment obtained from another taxonomic group,
- c) any fragment resulting or capable of resulting from the natural variability of the species, from which it is obtained,
- d) any fragment capable of resulting from the genetic engineering techniques applied to the reference fragment,
- e) any fragment, containing at least 30 contiguous nucleotides, encoding a peptide homologous or identical to the peptide encoded by the reference fragment,
- f) any fragment different from the reference fragment by insertion, deletion, substitution of at least one monomer, extension or shortening at at least

one of its ends; for example any fragment corresponding to the reference fragment flanked at least one of its ends by a nucleotide sequence not encoding a polypeptide.

5           The invention moreover relates to DNA or RNA fragments whose nucleotide sequence is identical, complementary, antisense or equivalent to any one of the following sequences:

10          - that starting at nucleotide 1232 and ending at nucleotide 2207 of SEQ ID No.1

              - that starting at nucleotide 1232 and ending at nucleotide 1825 of SEQ ID No.1

              - and that starting at nucleotide 1266 and ending at nucleotide 2207,

15          and especially the DNA or RNA fragments whose sequence has, for any succession of 30 contiguous monomers, at least 50%, preferably at least 60%, or better still at least 85% homology with any one of said sequences.

20          The subject of the invention is also a protein, called PTc100 by the applicant, having an apparent molecular mass of about 100 kDa, which is recognized by anti-Trypanosoma cruzi antisera, or an immunological equivalent of this protein, and fragments thereof. The 25 amino acid sequence of this protein is represented in the identifier sequence SEQ ID No.2.

              Immunological equivalent is understood to mean any polypeptide or peptide capable of being immunologically recognized by the antibodies directed against 30 said Ptcl00 protein.

              The invention also relates to any fragment of the Ptcl00 protein. A particular protein fragment has a

sequence starting at amino acid 323 and ending at amino acid 520 of the sequence defined in the identifier SEQ ID No.2, said fragment being specifically recognized by anti-Trypanosoma cruzi antisera; the invention also 5 relates to any immunological equivalent of said fragment.

The Ptcl00 protein and said protein fragments may contain modifications, especially chemical modifications, which do not alter their immunogenicity.

10 Moreover, the subject of the present invention is also an expression cassette which is functional especially in a cell derived from a prokaryotic or eukaryotic organism, and which allows the expression of DNA encoding the entire Ptcl00 protein or a fragment 15 thereof, in particular of a DNA fragment as defined above, placed under the control of elements necessary for its expression; said protein and said protein fragments being recognized by anti-Trypanosoma cruzi antisera.

20 Generally, any cell derived from a prokaryotic or eukaryotic organism can be used within the framework of the present invention. Such cells are known to persons skilled in the art. By way of examples, there may be mentioned cells derived from a eukaryotic 25 organism, such as the cells derived from a mammal, especially CHO (Chinese Hamster Ovarian) cells; insect cells; cells derived from a fungus, especially a unicellular fungus or from a yeast, especially of the strain Pichia, Saccharomyces, Schizosaccharomyces and 30 most particularly selected from the group consisting of Saccharomyces cerevisiae, Schizosaccharomyces pombe, Schizosaccharomyces malidevorans, Schizosaccharomyces

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IN INVENTION

sloofiae, Schizosaccharomyces octosporus. Likewise, among the cells derived from a prokaryotic organism, there may be used, without this constituting a limitation, the cells of a strain of Escherichia coli (E. coli) or enterobacterial cells. A large number of these cells are commercially available in collections, such as ATCC (Rockville, MA, USA) and AFRC (Agriculture & Food Research Council, Norfolk, UK). The cell may also be of the wild-type or mutant type. The mutations are described in the literature accessible to persons skilled in the art.

For the purposes of the present invention, an E. coli DH5a cell (marketed by the company CLONTECH under the reference: C2007-1) is used.

The expression cassette of the invention is intended for the production of the PTc100 protein or for fragments of said protein which are produced by the abovementioned E. coli cell, and which are recognized by human antisera. Such antisera are obtained from patients who have contracted a Trypanosoma cruzi infection recently or long ago, and contain immunoglobulins specifically recognizing PTc100. Of course, the PTc100 protein can also be recognized by other antibodies, such as for example monoclonal or polyclonal antibodies obtained by immunization of various species with the natural abovementioned protein, the recombinant protein or fragments or peptides thereof.

PTc100 protein is understood to mean the natural Trypanosoma cruzi cytoplasmic antigen, or the antigen produced especially by the genetic recombination techniques described in the present application, or any fragment or mutant of this antigen, provided

that it is immunologically reactive with antibodies directed against the PTc100 protein of this parasite.

Advantageously, such a protein has an amino acid sequence having a degree of homology of at least 5 70%, preferably of at least 85%, and most preferably of at least 95% relative to the sequence identified in the identifier SEQ ID No.2. In practice, such an equivalent can be obtained by deletion, substitution and/or addition of one or more amino acids of the native or 10 recombinant protein. It is within the capability of persons skilled in the art to perform, using known techniques, these modifications without affecting immunological recognition.

Within the framework of the present invention, 15 the PTc100 protein can be modified in vitro, especially by deletion or addition of chemical groups, such as phosphates, sugars or myristic acids, so as to enhance its stability or the presentation of one or several epitopes.

20 The expression cassette according to the invention allows the production of a PTc100 protein (having an amino acid sequence as specified above) and fragments of said protein, fused with an exogenous element which can help its stability, its purification, 25 its production or its recognition. The choice of such an exogenous element is within the capability of persons skilled in the art. It may be especially a hapten, an exogenous peptide or a protein.

The expression cassette according to the 30 invention comprises the elements necessary for the expression of said DNA fragment in the cell considered. "Elements necessary for the expression" is understood

to mean the elements as a whole which allow the transcription of the DNA fragment into messenger RNA (mRNA) and the translation of the latter into protein.

The present invention also extends to a vector 5 comprising an expression cassette according to the invention. This may be a viral vector and especially a vector derived from a baculovirus, more particularly intended for expression in insect cells, or an adenovirus-derived vector for expression in mammalian cells.

10 It may also be an autonomously replicating plasmid vector and in particular a multiplicative vector.

The present invention also relates to a cell derived from a prokaryotic or eukaryotic organism, 15 comprising an expression cassette, either in a form integrated in the cellular genome, or inserted in a vector. Such a cell was previously defined.

The subject of the present invention is also a process for preparing a PTc100 protein, or fragments of 20 said protein, according to which:

- (i) a cell derived from a prokaryotic or eukaryotic organism, comprising the expression cassette according to the invention, is cultured under appropriate conditions; and
- 25 (ii) the expressed protein derived from the abovementioned organism is recovered.

The present invention also relates to one or more peptides, whose amino acid sequence corresponds to a portion of the sequence of the PTc100 protein and 30 exhibiting, alone or as a mixture, a reactivity with the entire sera from individuals or animals infected with *Trypanosoma cruzi*.

The peptides can be obtained by chemical synthesis, lysies of the PTc100 protein or by genetic recombination techniques.

The invention also relates to monoclonal or 5 polyclonal antibodies obtained by immunological reaction of a human or animal organism to an immunogenic agent consisting of the natural or recombinant PTc100 protein and fragments thereof, or of a peptide, as defined above.

10 The present invention also relates to a reagent for the detection and/or monitoring of a Trypanosoma cruzi infection, which comprises, as reactive substance, a PTc100 protein as defined above, or fragments thereof, a peptide or a mixture of peptides as 15 defined above, or at least one monoclonal or polyclonal antibody as described above.

20 The above reagent may be attached directly or indirectly to an appropriate solid support. The solid support may be especially in the form of a cone, a tube, a well, a bead and the like.

The term "solid support" as used here includes all materials on which a reagent can be immobilized for use in diagnostic tests. Natural or synthetic materials, chemically modified or otherwise, can be 25 used as solid supports, especially polysaccharides such as cellulose-based materials, for example paper, cellulose derivatives such as cellulose acetate and nitrocellulose; polymers such as vinyl chloride, polyethylene, polystyrenes, polyacrylate or copolymers such as 30 polymers of vinyl chloride and propylene, polymers of vinyl chloride and vinyl acetate; styrene-based copoly-

mers, natural fibers such as cotton and synthetic fibers such as nylon.

Preferably, the solid support is a polystyrene polymer or a butadiene/styrene copolymer. Advantageously, the support is a polystyrene or a styrene-based copolymer comprising between about 10 and 90% by weight of styrene units.

The binding of the reagent onto the solid support may be performed in a direct or indirect manner.

Using the direct manner, two approaches are possible: either by adsorption of the reagent onto the solid support, that is to say by noncovalent bonds (principally of the hydrogen, Van der Walls or ionic type), or by formation of covalent bonds between the reagent and the support. Using the indirect manner, an "anti-reagent" compound capable of interacting with the reagent so as to immobilize the whole onto the solid support can be attached beforehand (by adsorption or covalent bonding) onto the solid support. By way of example, there may be mentioned an anti-PTc100 antibody, on the condition that it is immunologically reactive with a portion of the protein different from that involved in the reaction for recognizing the antibodies in the sera; a ligand-receptor system, for example by grafting onto the PTc100 protein a molecule such as a vitamin, and by immobilizing onto the solid phase the corresponding receptor (for example the biotin-streptavidin system). Indirect manner is also understood to mean the preliminary grafting or fusion by genetic recombination of a protein, or a fragment of this protein, or of a polypeptide, to one end of the

PTc100 protein, and the immobilization of the latter onto the solid support by passive adsorption or covalent bonding of the protein or of the polypeptide grafted or fused.

5       The invention also relates to a process for the detection and/or monitoring of a *Trypanosoma cruzi* infection in a biological sample, such as a blood sample from an individual or an animal likely to have been infected with *Trypanosoma cruzi*, characterized in  
10      that said sample and a reagent as defined above are placed in contact, under conditions allowing a possible immunological reaction, and the presence of an immune complex with said reagent is then detected.

15      By way of non-limiting example, there may be mentioned the sandwich-type detection process in one or more stages, as especially described in patents FR 2,481,318 and FR 2,487,983, which consists in reacting a first monoclonal or polyclonal antibody specific for a desired antigen, attached onto a solid support,  
20      with the sample, and in revealing the possible presence of an immune complex thus formed using a second antibody labelled by any appropriate marker known to persons skilled in the art, especially a radioactive isotope, an enzyme, for example peroxidase or alkaline phosphatase and the like, using so-called competition  
25      techniques well known to persons skilled in the art.

30      The subject of the invention is also an active immunotherapeutic composition, especially a vaccinal preparation, which comprises as active ingredient, a natural or recombinant PTc100 protein or fragments thereof, or the peptides identified above, the active ingredient being optionally conjugated with a pharma-

aceutically acceptable carrier, and optionally an excipient and/or an appropriate adjuvant.

The present invention also covers a pharmaceutical composition intended for the treatment or for 5 the prevention of a *Trypanosoma cruzi* infection in man or in an animal, comprising a therapeutically effective quantity of an expression cassette, a vector, a cell derived from a prokaryotic or eukaryotic organism as defined above, a PTc100 protein according to the 10 invention, or fragments thereof, or an antibody of the invention.

The subject of the present invention is also probes and primers specific for *T. cruzi*, and their uses in diagnostic tests.

15       The term probe as used in the present invention refers to a DNA or RNA containing at least one strand having a nucleotide sequence which allows hybridization to nucleic acids having a nucleotide sequence as represented in the identifier SEQ ID No.1, or a complementary or antisense sequence, or a sequence equivalent to said sequence, and especially a sequence having, for any succession of 5 to 100 contiguous monomers, at least 50%, preferably at least 60%, or even better at least 85% homology with SEQ ID No.1, with fragments 20 thereof, or with a synthetic oligonucleotide allowing such a hybridization, nonmodified or comprising one or more modified bases such as inosine, 5-methyldeoxycytidine, deoxyuridine, 5-dimethylaminodeoxyuridine, 2,6-diaminopurine, 5-bromodeoxyuridine or any other modified base. Likewise, these probes may be modified at 25 the level of the sugar, namely the replacement of at least one deoxyribose with a polyamide (P. E. NIELSEN 30

et al. (1991) (13)), or at the level of the phosphate group, for example its replacement with esters, especially chosen from esters of diphosphate, of alkyl and arylphosphonate and of phosphorothioate.

5       The probes may be much shorter than the sequence identified in the identifier SEQ ID No.1. In practice, such probes comprise at least 5 monomers, advantageously from 8 to 50 monomers, having a hybridization specificity, under defined conditions, to form  
10 a hybridization complex with DNA or RNA having a nucleotide sequence as defined above.

A probe according to the invention can be used for diagnostic purposes as capture and/or detection probe, or for therapeutic purposes.

15       The capture probe can be immobilized on a solid support by any appropriate means, that is to say directly or indirectly, for example by covalent bonding or passive adsorption.

20       The detection probe is labelled by means of a marker chosen from radioactive isotopes, enzymes especially chosen from peroxidase and alkaline phosphatase, and those capable of hydrolyzing a chromogenic, fluorogenic or luminescent substrate, chromophoric chemical compounds, chromogenic, fluorogenic or  
25       luminescent compounds, nucleotide base analogs, and biotin.

The probes of the present invention which are used for diagnostic purposes can be used in any known hybridization techniques, and especially the so-called  
30 "Dot-Blot" technique (Maniatis et al. (1982) (14)), Southern Blotting technique (Southern E. M. (1975) (15)), Northern Blotting technique, which is a

technique identical to the Southern Blotting technique but which uses RNA as target, sandwich technique (Dunn A.R. et al. (1977) (16)). Advantageously, the sandwich technique is used which comprises a specific capture probe and/or a specific detection probe, it being understood that the capture probe and the detection probe must have a nucleotide sequence which is at least partially different.

Another application of the invention is a therapeutic probe for treating infections due to Trypanosoma cruzi, said probe being capable of hybridizing in vivo with the DNA or RNA of the parasite to block the trans-lation and/or transcription and/or replication pheno-mena.

A primer is a probe comprising 5 to 30 monomers, having a hybridization specificity, under predefined conditions, for the initiation of an enzymatic polymerization, for example in an amplification technique such as PCR (Polymerase Chain Reaction), in an elongation process such as sequencing, in a reverse transcription method and the like.

A preferred probe or primer will contain a nucleotide sequence chosen from the sequences SEQ ID No.7, SEQ ID No.8, SEQ ID No.9, SEQ ID No.10, SEQ ID No.12.

The invention also relates to a reagent for detecting and/or identifying Trypanosoma cruzi in a biological sample, comprising at least one probe as defined above, and in particular a capture probe and a detection probe, either or both corresponding to the above definition.

The invention therefore provides a process for selectively detecting and/or for identifying *Trypanosoma cruzi* in a biological sample, according to which the RNA, extracted from the parasite and optionally denatured, or the DNA, denatured extract, or the DNA obtained from reverse transcription of the RNA, is exposed to at least one probe as defined above and the hybridization of said probe is detected.

The invention will be understood more clearly upon reading the detailed description below which is made with reference to the accompanying figures in which:

Figure 1 represents the restriction map of the Tc100 gene, which map is deduced by Southern blotting of different fragments obtained after digestion of *Trypanosoma cruzi* DNA with restriction endonucleases.

Figure 2 is a schematic representation of the three overlapping regions of the Tc100 cDNA. The numbered arrows represent the oligonucleotides used as primers for the PCR amplification.

Example 1: Isolation of the Tc50 clone

An expression library was constructed from Trypanosoma cruzi genomic DNA fragments. The *T. cruzi*, strain G (YOSHIDA. N., (1983) (17)), DNA isolated from the metacyclic trypomastigote stage was digested with the enzyme DNase I. After selection of the fragments according to their size, they were ligated to synthetic EcoRI adaptors and cloned into the EcoRI site of lambda gt11 vector DNA (Young and Davis, 1983 (3); Cotrim et al., 1990) (4).

The clone, called Tc50 by the applicant, was isolated from the library by immunological screening with the aid of a mixture of sera from patients suffering from the chronic phase of the Chagas disease.

5 The Tc50 phage clone was purified, amplified and the insert was detected by the PCR ("Polymerase Chain Reaction") technique with the aid of the primers:

SEQ ID No.3 5' (GGTGGCGACGACTCCTGGAGCCCG) 3' 24,

and

10 SEQ ID No.4 5' (TTGACACCAGACCAACTGGTAATG) 3' 24 corresponding respectively to the nucleotide sequence of the left and right arms of the lambda gt11 phage DNA.

15 The 594 base pairs (bp) Tc50 DNA fragment, after EcoRI digestion, was subcloned into the expression vector pGEX (Pharmacia) linearized with EcoRI. The sequencing of the Tc50 clone DNA was carried out in this same vector with the aid of specific primers situated in 3' and 5' of the cloning site of 20 pGEX, according to the chain termination technique (Sanger et al., 1977 (5)) and according to the manufacturer's procedure (USB-Amersham).

25 The nucleotide sequence of the 594 bp Tc50 fragment as well as its deduced amino acid sequence (198 aa) are represented in the identifiers SEQ ID No.1 and SEQ ID No.2, respectively. The nucleotide sequence of the 594 bp Tc50 fragment starts at nucleotide (nt) 1232 and ends at nucleotide 1825. The corresponding amino acid sequence starts at amino acid 323 and ends 30 at amino acid 520 of SEQ ID No.2.

Example 2: Expression of the Tc50 clone in  
Escherichia coli

The construct pGEX-Tc50 (198 aa) synthesizes,  
5 in the bacterium DH5alpha, a protein fused with GST  
("Glutathione S Transferase"), with an apparent mole-  
cular mass of 50 kDa, which is detected by SDS-PAGE  
polyacrylamide gel electrophoresis (SDS: sodium dodecyl  
sulfate) (Laemmli, 1970 (6)). The reactivity of the  
10 protein towards chagasic human sera was confirmed by  
the Western blotting technique (Towbin et al., 1979  
(7)) with the aid of the same mixture of chronic phase  
chagasic sera which is used for screening the lambda  
gt11 library.

15 The soluble fraction of the recombinant GST-  
Tc50 protein obtained after lysis of the bacterial  
extracts by ultrasound was purified by affinity  
chromatography on a glutathione agarose column (Sigma),  
according to the method of Smith and Johnson, (1988)  
20 (8).

The antigenic properties of the recombinant  
GST-Tc50 antigen were tested by ELISA (Voller et al.,  
1975 (9)). For that, microtiter plates (Maxisorp (trade  
name), nunc) were sensitized with 100 ng/ml of GST-Tc50  
25 antigen in 100 mM NaHCO3 (pH 9.6). After incubation  
with the patients' sera, the immune complexes were  
detected with the aid of a peroxidase-coupled anti-  
human IgG goat serum.

The results are presented in the accompanying  
30 table and show that the entire chagasic human sera  
tested react specifically with the recombinant protein.  
No cross-reactivity was observed on 7 sera from

patients suffering from cutaneous or visceral leishmaniosis.

5       Example 3: Identification of the native T.  
cruzi protein having the antigenic determinants of the  
Tc50 clone

The detection of the native *T. cruzi* protein was performed after immunopurification of a mixture of 10 chagasic human sera on the corresponding recombinant protein called PTc50 by the applicant.

The eluate of monospecific polyclonal antibodies which is obtained was used as probe, in Western blotting, on total protein extracts of different stages 15 of the parasite. The selected antibodies specifically reacted with a protein of apparent molecular mass 100 kDa, called PTc100 by the applicant, which is expressed in all the tested strains of the parasite.

20       Example 4: Molecular analysis of the Tc100 gene  
-Southern blots

In order to establish the restriction map of the Tc100 gene (Figure 1), the *T. cruzi*, strain G, nuclear DNA was digested with different restriction 25 endonucleases (BamHI, EcoRI, HindIII, PstI, PvuII, SacI, BamHI/EcoRI, BamHI/PvuII, EcoRI/HindIII, EcoRI/PstI, EcoRI/PvuII, EcoRI/SacI, PstI/SacI, PstI/PvuII, PvuII/SacI, PvuII/HindIII), separated on agarose gel and then transferred onto a nylon filter 30 according to the Southern technique. The Southern blot hybridization was performed with the 594 bp Tc 50 DNA, which is a fragment of the Tc100 DNA described above,

radiolabelled with  $^{32}\text{P}$  by random incorporation (Amersham).

- Cloning of a 3500 bp Tc100 genomic fragment

5 According to the results obtained by Southern blotting, the *T. cruzi*, strain G, genomic DNA was digested with the enzyme EcoRI and then separated on agarose gel. The EcoRI restriction fragments of about 3500 bp (Figure 1) were cloned into the vector lambda 10 gt10 (Huynh et al., 1984 (10)) linearized by EcoRI. The phage clone containing the 3500 bp Tc100 genomic insert was isolated with the aid of the 594 bp radiolabelled probe described above. A 1041 bp fragment situated in the 3' region of the 3500 bp Tc100 genomic insert was 15 sequenced. This sequencing was carried out gradually with the aid of the following primers:

SEQ ID No.5 5' (TCGGGCAGTGACGCGCG) 3' 18

SEQ ID No.6 5' (CTTATGAGTATTCTCCAGGGTA) 3' 24

20 The primer SEQ ID No.5 is situated in the previously sequenced portion of the 594 bp Tc50 fragment. The primer SEQ ID No.6 corresponds to the lambda gt10 phage primer.

25 This 1041 bp fragment, which starts at nucleotide 1403 and ends at nucleotide 2443 of SEQ ID No.1, has an open reading frame in phase with the sequence of the 594 bp Tc50 fragment.

Example 5: Cloning of the Tc100 cDNA

The cDNA was synthesized from total RNA from *T. cruzi*, strain G, epimastigots. The Tc100 cDNA was  
5 amplified by the PCR technique in three different fragments: a fragment A corresponding to the 5' region of 1459 bp, a fragment B corresponding to the central region of 942 bp, a fragment C corresponding to the 3' region of 1406 bp of the Tc100 cDNA, as schematically  
10 represented in Figure 2.

- Cloning of fragment A of the Tc100 cDNA

The total cDNA synthesized by AMV ("avian myeloblastosis virus") reverse transcriptase, with the  
15 aid of random hexanucleotides (Boehringer Mannheim), was amplified, by PCR, using the following pair of primers:

SEQ ID No.7 5' (AACGCTATTATTAGAACAGTT) 3' 21, and  
SEQ ID No.8 5' (TGCAGCAGCGGCAGAAGT) 3' 18

20

SEQ ID No.7 corresponds to a portion of the consensus sequence of 35 nucleotides present in 5' of the mRNAs in trypanosomatides and called "spliced leader" (Parsons et al. 1984 (11)).

25

SEQ ID No.8 corresponds to the sequence complementary to a portion of the predetermined sequence of the 594 bp fragment, and starts at nucleotide 1442 and ends at nucleotide 1459 of SEQ ID No.1, according to the coding strand numbering.

30

After verification by Southern blotting with the aid of the radiolabelled 594 bp probe previously described, the 1459 bp cDNA fragment corresponding to

the 5' region of Tc100 was cloned into the plasmid called pCRII (trade name) (Invitrogen), and sequenced. The sequence represented in the identifier SEQ ID No.1 starts at nucleotide 1 and ends at nucleotide 1459.

5

- Cloning of fragment B of the Tc100 cDNA

The T. cruzi total cDNA was amplified by PCR with the aid of the primers:

SEQ ID No.9 : 5' (CAGCCGACGGTAGCTGCGTCCT) 3' 22

10 and

SEQ ID No.10: 5' (ACATAATGGCCTCGTTCACAC) 3' 21

15 The sequence ID No.9 which corresponds to a portion of the 594 bp predetermined sequence of the Tc100 gene starts at nucleotide 1266 and ends at nucleotide 1287 of SEQ ID No.1.

20 The sequence SEQ ID No.10 corresponds to the sequence complementary to a portion of the 1041 bp previously described sequence of the Tc100 gene. This sequence SEQ ID No.10 starts at nucleotide 2187 and ends at nucleotide 2207 of SEQ ID No.1, according to the coding strand numbering.

25 The fragment obtained, 942 bp in length, was cloned into the plasmid pCRII and sequenced. The sequence represented in the identifier SEQ ID No.1 starts at nucleotide 1266 and ends at nucleotide 2207.

- Cloning of fragment C of the Tc100 cDNA

30 In order to isolate the 3' portion of the Tc100 cDNA, the T. cruzi total cDNA was synthesized with the aid of the adaptor oligo(dT)<sub>16</sub> hybrid primer.

SEQ ID No.11 5' (GACTCGCTGCAGATCGATTTTTTTTTTT) 3' 34  
according to the RACE ("Rapid Amplification of cDNA  
Ends") procedure (Frohman et., 1988 (12)).

The 3' region of the Tc100 cDNA was amplified  
5 using the adaptor primer and the following pair of  
primers:

SEQ ID No.12: 5' (CGAAGAGACCATGAACAACTT) 3' 21

and

SEQ ID No.13: 5' (GACTCGCTGCAGATCGAT) 3' 18

10

The sequence SEQ ID No.12 corresponds to a  
portion of the previously described 1041 bp sequence of  
the Tc100 gene, starting at nucleotide 1997 and ending  
at nucleotide 2017.

15 The sequence SEQ ID No.11 corresponds to the  
arbitrary sequence of the adaptor represented in SEQ ID  
No.11.

After checking by Southern blotting using the  
1041 bp radiolabelled fragment previously described,  
20 the 3' fragment of the Tc100 cDNA, 1423 bp long, was  
cloned into pCRII and sequenced. The sequence  
represented in the identifier SEQ ID No.1 starts at  
nucleotide 1997 and ends at nucleotide 3402.

The Tc100 complete cDNA, 3402 bp in size, was  
25 completely sequenced. It has a 2745 bp open reading  
frame and the deduced amino acid sequence is 915. The  
methionine codon is in position 266 and the stop codon  
in position 3011.

The Trypanosoma cruzi Tc100 gene encodes the  
30 new PTc100 protein of theoretical molecular mass 100  
kDa.

Of course, since the DNA sequence of the gene has been fully identified, it is possible to produce the corresponding DNA solely by chemical synthesis, and then to insert the DNA into commercially available DNA vectors, using known techniques from the technology relating to genetic recombination.

TABLE

Disease	Sera	OD (492nm) detection threshold = 0.320
CHAGAS DISEASE	1	1.358 (+)
	2	1.278 (+)
	3	0.328 (+)
	4	0.404 (+)
	5	1.378 (+)
	6	1.059 (+)
	7	0.895 (+)
	8	1.791 (+)
	9	1.635 (+)
	10	1.427 (+)
	11	1.009 (+)
	12	1.743 (+)
	13	0.530 (+)
	14	1.035 (+)
	15	0.461 (+)
CUTANEOUS LEISHMANIOSIS	16	0.291 (-)
VISCERAL LEISHMANIOSIS (Kala azar)	17	0.071 (-)
	18	0.081 (-)
	19	0.279 (-)
	20	0.098 (-)
	21	0.067 (-)
	22	0.125 (-)

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: PARANHOS-BACCALA, Glauzia  
LESENECHAL, Mylene  
JOLIVET, Michel

(ii) TITLE OF INVENTION: NEW TRYPANOSOMA CRUZI ANTIGEN, AND  
GENE ENCODING THE LATTER; THEIR APPLICATION TO THE  
DETECTION OF CHAGAS DISEASE

(iii) NUMBER OF SEQUENCES: 13

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Oliff & Berridge  
(B) STREET: 700 South Washington Street, Suite 300  
(C) CITY: Alexandria  
(D) STATE: Virginia  
(E) COUNTRY: U.S.A.  
(F) ZIP: 22314

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: US 08/480,917  
(B) FILING DATE: 07-JUN-1995  
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Berridge, William P.  
(B) REGISTRATION NUMBER: 30,024  
(C) REFERENCE/DOCKET NUMBER: WPB 36400

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 703-836-6400  
(B) TELEFAX: 703-836-2787

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 3402 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AACGCTATTA TTAGAACAGT TTCTGTACTA TATTGTCATT TGGGGAGGGG GGAAAGGGGG	60
GAAGTACTTG CCGTTTGTG TGGGTGACGA GACAACACAC ATCGAGCGGG AAGAAAAAAA	120
AAAAGGAAAT AAATTAAATT AAATTATTTG TTCTTTGAAT AGGCAAAGAA GAAGAAGAAG	180

AAAAGGTGCG	GGGGAGGGAG	GAGAAAGCGA	CACACACACA	AAAAAAAAAA	AAGGAATTGC	240
GGAAATAACA	ACGCAAGGCG	CGGACATGAC	CGTACGGTG	GATTTGTTCA	ATCATGCGAA	300
GCCGAGCAAC	AATGAGGGCC	CCGTGTGGTC	TGTGGACGCC	GCGACATTAA	ACGAGGTGCC	360
TGAGGCGCAG	CGTGTGCTGG	CGGATTCGCA	GTTTATCTT	GCCTACACCA	TGAAGCGGCG	420
TCACGTGCTG	CGTGTGGTGA	AGCGCTCGAA	CCTTTGAAG	GGCACCGTGC	GGGCACACTC	480
AAAGCCCATT	CATGCGGTGA	AGTTTGTGAA	TTACCGCAGT	AACGTCGCAG	CATCGGCTGG	540
GAAGGGGGAG	TTCTTCGTGT	GGGTTGTGAC	GGATGAAACG	GAGGCGAGCA	ACGGCAAGCC	600
GGATCTCGCA	GCCCCCCTCA	CAGTGAAGGT	GTACTTTAACG	CTTCAGGATC	CTGTCACAAT	660
TCCATGCTTT	TCTTCTTTA	TCAACGCCGA	GAGTCAGCGG	CCTGATCTGC	TTGTCCTTTA	720
CGAAACGCAG	GCGGCAATTG	TTGACAGCTC	CTCCCTCATT	GAGCGCTTTG	ACGTGGAATC	780
ACTGGAGGCA	ACACTACAGC	GGAATTGCAC	AACCCCTGCAG	ACCCTGACTC	AACCGGTTAG	840
TGAGAACAGT	TTATGCTCCG	TTGGCTCTGG	CGGATGGTTC	ACCTTTACCA	CGGAACCAAAC	900
AATGGTAGCG	GCATGCACAT	TACGAAACCG	CAGCACTCCA	TCATGGCGT	GTTGCGAGGG	960
TGAGCCAGTG	AAGGCATTGC	ATCTCCTTGA	CGCAACCGTT	GAGGAAAATG	TCAGTGTCT	1020
CGTGGCCGCA	TCTACAAAAG	GGGTGTACCA	ATGGCTCCTT	ACGGGTGTAG	CAGAACCAAA	1080
CTTGTGCGC	AAGTTTGTCA	TTGATGGATC	TATTGTCGCG	ATGGAAAGCT	CACGAGAAAC	1140
TTTGCCGTG	TTTGACGACA	GGAAGCAGCT	GGCGCTGGTC	AACATGCATT	CCCCTCATAA	1200
CTTTACCTGC	ACACACTACA	TGATGCCTTG	TCAGGTACAG	CGTAACGGCT	TTTGCTTC	1260
TCGTACAGCC	GACGGTAGCT	GCGTCCTGGC	TGACATGTG	ATTGATTGA	CGATCTTCCA	1320
TCTCCGGTCC	TCCCGCAGGG	AAGAACAGCA	GCCAGGCCAA	AAAACATCGG	TAGTGGCGAC	1380
GGCGAAACCG	GGGTGTGTGT	CCTCGGGCAC	TGACGCGGCG	AGTAGCAGTC	ATACCAATAC	1440
GACTTCTGCC	GCTGCTGCAT	CCCCTGCATC	ACCCCCCTGTT	TCAGCGCCAG	CCAAGGCAGC	1500
CGCGCCTCCT	GCCGCGGCCGC	GATCGGCTGA	GCCGCACGTG	GGGAGCAAGA	TCATTGCTAA	1560
TCTAGTGAAT	CAGCTGGGA	TTAATGTCAC	CCAAAGGAGC	GTCGTAGCA	CTGGAGCGCC	1620
GGCCACGACG	AGGTCTACGG	CGGTGACGTC	CACGACTACC	GCCCCGCAGC	GAACAAGTCC	1680
ATACGGGCAC	AATGGCCGAC	CTGTACGGC	TGGATTGGTG	GCAGCTAATA	GTGGTGCCAG	1740
CGCGGCCCTCG	TCTCCCACAG	CCGCGGCCGA	ACCAACAGGA	GAAGAAAAGG	CCTCCGCGGC	1800
ATGTGAAACG	AGCTCCGTGG	CGATAAAATGC	GACACGCCCG	GCGCTTCACA	ACGCCTCTCT	1860
CCCGCAGGCG	CCAACGGATG	GCCTTTGGC	GGCAGCAGTA	TACCAAGTCGG	AGGGCGAGGT	1920
TCATCAGTCG	CTGGAGCGGC	TGGAGTCCGT	CATAACCAAC	ACGTCTCGGG	TTCTGAAGTT	1980
GCTCCCTGAC	ACCATTGAA	GAGACCATGA	ACAACTTCTG	AATCTGGTT	TAGAGGCACA	2040

GATGACAGAG CTGCAGCAGA GCCGTCAAAC ACCGCAAACA CAGCCGAGAG ACACAAGCTC	2100
CGCGAAATCA TCCGTGTTTG AGACGTACAC CCTTGTTCCTC ATTGCCGGATT CCCTCTCTCG	2160
CAACATCACG AAGGGGGTGA AGCGTGGTGT GAACGAGGCC ATTATGTTGC ATCTCGACCA	2220
TGAGGTGCGG CACGCCATAG GGAACCGGCT TCGGCAAACA CAAAAGAACAA TCATCAAGAG	2280
CCGCCTCGAT GAAGCGTTGA AGGAAAGCAC TACACAGTTT ACGGCTCAAT TGACGCAAAC	2340
GGTGGAGAAT CTGGTGAAGC GCGAGCTTGC CGAGGTGCTT GGTAGCATCA ACGGCTCCCT	2400
CACTTCTCTC GTGAAGGAAA ATGCCTCATT ACAGAAAGAG TTGAATTCCA TAATGTCTAG	2460
TGGGGTGTG GATGAAATGC GTCGTATGCG GGAAGAGCTG TGCACATTGC GAGAGTCCGT	2520
TGCGAAGCGG AAGGCAACAA TGCCAGATTC TTCTCTTCAC GCCACGAGCT CCTTTCAAGG	2580
AAGAAGGTCT GCGCCCGAGA CAATTCTTGC AACCGCGTTA TCGATGGTGC GAGAGCAGCA	2640
ATACCGTCAG GGACTGGAAT ACATGTTGAT GGCTCAGCAG CCCTCTCTCC TCCTGCGGTT	2700
CCTCAGCATA CTTACAAGGG AAAACGAAAA CGCCTACTCG GAACTTATTG AAAATGTAGA	2760
GACGCCGAAT GACGTGTGGT GTTCGGTTCT GTTGCAACTC ATAGAGGCCG CGGCGACCGA	2820
GGCTGAGAAG GAGGTGGTTG TTGGCGTCGC CATTGATATT CTCTCCGAGC GCGATCAAAT	2880
TGCTCAGAAC GGCGCACTCG GCTCGAAACT CACCACCGCC ATGCGAGCCT TTGAGCGACA	2940
GGCAAGGTCG GAGACAACGA GCAGGTCATT CTTGCAATGC CTGAAGAACCTGGAAAAGCT	3000
TCTGCAATCA TGATAATAAA AAGAACTCAA CGAATACAGT TGTTGATTAT TAAGGAAGGG	3060
AAAAGAGAGA AAGAGAGAGA GAGAGAGAGA AATGTAATGG GCGTTTAGTT ACGGTAGAAA	3120
AAAAACGTGT GGATAAGAAG GAGGGGTTTT GTGTGCGACC AGGAATTACT GGGGAACGCT	3180
GCTACACGGC GGAATCGACC ATTTTATTAT TATTATTATT GTCTTTAGTA TTATGTTTTT	3240
TCTTGTGTGT GTGTGTGTGT GTTTGTGTGT GTGCGGTTAT TTTGTATCCG TTTGCTCCCG	3300
CCCCTGCCCG CCATCACCCG AGGAGAAAGT AGAATAAGAC ACATACGATT GTTGTGTTTG	3360
TTATCCTTAA AAGGAAGAGA GACCAAAAAAA AAAAAAAAAAA AA	3402

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 915 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "protein"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Thr Val Thr Val Asp Leu Phe Asn His Ala Lys Pro Ser Asn Asn  
1 5 10 15

Glu Gly Arg Val Trp Ser Val Asp Ala Ala Thr Phe Asn Glu Val Pro  
20 25 30

Glu Ala Gln Arg Val Leu Ala Asp Ser Gln Phe Tyr Leu Ala Tyr Thr  
35 40 45

Met Lys Arg Arg His Val Leu Arg Val Val Lys Arg Ser Asn Leu Leu  
50 55 60

Lys Gly Thr Val Arg Ala His Ser Lys Pro Ile His Ala Val Lys Phe  
65 70 75 80

Val Asn Tyr Arg Ser Asn Val Ala Ala Ser Ala Gly Lys Gly Glu Phe  
85 90 95

Phe Val Trp Val Val Thr Asp Glu Thr Asp Ala Ser Asn Gly Lys Pro  
100 105 110

Asp Leu Ala Ala Arg Leu Thr Val Lys Val Tyr Phe Lys Leu Gln Asp  
115 120 125

Pro Val Thr Ile Pro Cys Phe Ser Phe Phe Ile Asn Ala Glu Ser Gln  
130 135 140

Arg Pro Asp Leu Leu Val Leu Tyr Glu Thr Gln Ala Ala Ile Leu Asp  
145 150 155 160

Ser Ser Ser Leu Ile Glu Arg Phe Asp Val Glu Ser Leu Glu Ala Thr  
165 170 175

Leu Gln Arg Asn Cys Thr Thr Leu Arg Thr Leu Thr Gln Pro Val Ser  
180 185 190

Glu Asn Ser Leu Cys Ser Val Gly Ser Gly Gly Trp Phe Thr Phe Thr  
195 200 205

Thr Glu Pro Thr Met Val Ala Ala Cys Thr Leu Arg Asn Arg Ser Thr  
210 215 220

Pro Ser Trp Ala Cys Cys Glu Gly Glu Pro Val Lys Ala Leu His Leu  
225 230 235 240

Leu Asp Ala Thr Val Glu Glu Asn Val Ser Val Leu Val Ala Ala Ser  
245 250 255

Thr Lys Gly Val Tyr Gln Trp Leu Leu Thr Gly Val Ala Glu Pro Asn  
260 265 270

Leu Leu Arg Lys Phe Val Ile Asp Gly Ser Ile Val Ala Met Glu Ser  
275 280 285

Ser Arg Glu Thr Phe Ala Val Phe Asp Asp Arg Lys Gln Leu Ala Leu  
290 295 300

Val Asn Met His Ser Pro His Asn Phe Thr Cys Thr His Tyr Met Met  
305 310 315 320

Pro Cys Gln Val Gln Arg Asn Gly Phe Cys Phe Asn Arg Thr Ala Asp  
325 330 335

Gly Ser Cys Val Leu Ala Asp Met Ser Asn Arg Leu Thr Ile Phe His  
340 345 350

Leu Arg Cys Ser Arg Arg Glu Glu Gln Gln Pro Gly Gln Lys Thr Ser  
355 360 365

Val Val Ala Thr Ala Lys Pro Gly Cys Val Ser Ser Gly Thr Asp Ala  
370 375 380

Ala Ser Ser Ser His Thr Asn Thr Thr Ser Ala Ala Ala Ala Ser Pro  
385 390 395 400

Ala Ser Pro Pro Val Ser Ala Pro Ala Lys Ala Ala Ala Pro Pro Ala  
405 410 415

Ala Ala Arg Ser Ala Glu Pro His Val Gly Ser Lys Ile Ile Ala Asn  
420 425 430

Leu Val Asn Gln Leu Gly Ile Asn Val Thr Gln Arg Ser Val Val Ser  
435 440 445

Thr Gly Ala Pro Ala Thr Thr Arg Ser Thr Ala Val Thr Ser Thr Thr  
450 455 460

Thr Ala Pro Gln Arg Thr Ser Pro Tyr Gly His Asn Gly Arg Pro Val  
465 470 475 480

Thr Ala Gly Leu Val Ala Ala Asn Ser Gly Ala Ser Ala Ala Ser Ser  
485 490 495

Pro Thr Ala Ala Ala Lys Pro Thr Gly Glu Glu Lys Ala Ser Ala Ala  
500 505 510

Cys Glu Thr Ser Ser Val Ala Ile Asn Ala Thr Arg Pro Ala Leu His  
515 520 525

Asn Ala Ser Leu Pro Gln Ala Pro Thr Asp Gly Val Leu Ala Ala Ala  
530 535 540

Val Tyr Gln Ser Glu Gly Glu Val His Gln Ser Leu Glu Arg Leu Glu  
545 550 555 560

Ser Val Ile Thr Asn Thr Ser Arg Val Leu Lys Leu Leu Pro Asp Thr  
565 570 575

Ile Arg Arg Asp His Glu Gln Leu Leu Asn Leu Gly Leu Glu Ala Gln  
580 585 590

Met Thr Glu Leu Gln Gln Ser Arg Pro Thr Pro Gln Thr Gln Pro Arg  
595 600 605

Asp Thr Ser Ser Ala Lys Ser Ser Val Phe Glu Thr Tyr Thr Leu Val  
610 615 620

Leu Ile Ala Asp Ser Leu Ser Arg Asn Ile Thr Lys Gly Val Lys Arg  
625 630 635 640

Gly Val Asn Glu Ala Ile Met Leu His Leu Asp His Glu Val Arg His  
645 650 655

Ala Ile Gly Asn Arg Leu Arg Gln Thr Gln Lys Asn Ile Ile Lys Ser  
660 665 670

Arg Leu Asp Glu Ala Leu Lys Glu Ser Thr Thr Gln Phe Thr Ala Gln  
675 680 685

Leu Thr Gln Thr Val Glu Asn Leu Val Lys Arg Glu Leu Ala Glu Val  
690 695 700

Leu Gly Ser Ile Asn Gly Ser Leu Thr Ser Leu Val Lys Glu Asn Ala  
705 710 715 720

Ser Leu Lys Lys Glu Leu Asn Ser Ile Met Ser Ser Gly Val Leu Asp  
725 730 735

Glu Met Arg Arg Met Arg Glu Glu Leu Cys Thr Leu Arg Glu Ser Val  
740 745 750

Ala Lys Arg Lys Ala Thr Met Pro Asp Ser Ser Leu His Ala Thr Ser  
755 760 765

Ser Phe Gln Gly Arg Arg Ser Ala Pro Glu Thr Ile Leu Ala Thr Ala  
770 775 780

Leu Ser Met Val Arg Glu Gln Gln Tyr Arg Gln Gly Leu Glu Val Met  
785 790 795 800

Leu Met Ala Gln Gln Pro Ser Leu Leu Leu Arg Phe Leu Ser Ile Leu  
805 810 815

Thr Arg Glu Asn Glu Asn Ala Tyr Ser Glu Leu Ile Glu Asn Val Glu  
820 825 830

Thr Pro Asn Asp Val Trp Cys Ser Val Leu Leu Gln Leu Ile Glu Ala  
835 840 845

Ala Ala Thr Glu Ala Glu Lys Glu Val Val Val Gly Val Ala Ile Asp  
850 855 860

Ile Leu Ser Glu Arg Asp Gln Ile Ala Gln Asn Gly Ala Leu Gly Ser  
865 870 875 880

Lys Leu Thr Thr Ala Met Arg Ala Phe Glu Arg Gln Ala Arg Ser Glu  
885 890 895

Thr Thr Ser Arg Ser Phe Leu Gln Cys Leu Lys Asn Leu Ile Lys Leu  
900 905 910

Leu Gln Ser  
915

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 24 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
  - (A) DESCRIPTION: /desc = "phage DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GGTGGCGACG ACTCCTGGAG CCCG

24

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "phage DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TTGACACCAG ACCAACTGGT AATG

24

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TCGGGCACTG ACGCGGCG

18

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "phage lambda gt10 DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CTTATGAGTA TTTCTTCCAG GGTA

24

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

AACGCTATTA TTAGAACAGT T

21

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 18 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

TGCAGCAGCG GCAGAAAGT

18

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 22 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CAGCCGACGG TAGCTGCGTC CT

22

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

ACATAATGGC CTCGTTCACAC

21

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 34 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GACTCGCTGC AGATCGATTT TTTTTTTTTT TTTT

34

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 21 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CGAAGAGACC ATGAACAACT T

21

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 18 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid  
(A) DESCRIPTION: /desc = "DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GACTCGCTGC AGATCGAT

18

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CLAIMS

1. A synthetic or isolated nucleic acid fragment which comprises a nucleotide sequence that is identical, complementary, antisense or equivalent to a first sequence starting at nucleotide 1232 and ending at nucleotide 1825 of SEQ ID NO:1.

2. The nucleic acid fragment according to claim 1, wherein said nucleotide sequence is identical, complementary, antisense or equivalent to a second sequence starting at nucleotide 1232 and ending at nucleotide 2207 of SEQ ID NO:1.

3. The nucleic acid fragment according to claim 1, wherein an at least 30 nucleotide segment of said nucleotide sequence is at least 50% homologous with a correspondingly long segment of the sequence identified in SEQ ID NO:1.

4. The nucleic acid fragment according to claim 2, wherein an at least 30 nucleotide segment of said nucleotide sequence is at least 50% homologous with a correspondingly long segment of the sequence identified in SEQ ID NO:1.

5. A probe for identifying *Trypanosoma cruzi*, said probe comprising a nucleotide sequence that is hybridizable to at least a segment of a nucleic acid according to claim 1.

6. The probe according to claim 5, wherein said probe comprises 5 to 100 nucleotides.

7. The probe according to claim 5, wherein said probe comprises 8 to 50 nucleotides.

8. A primer for amplifying a nucleotide sequence, said primer comprising a nucleotide sequence that allows hybridization to at least a segment of a nucleic acid according to claim 1.

5 9. The primer according to claim 8, wherein said nucleotide sequence comprises at least five nucleotides.

10. The primer according to claim 9, wherein said nucleotide sequence is selected from the group consisting of SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10  
10 and SEQ ID NO:12.

11. A reagent for detecting or identifying Trypanosoma cruzi in a biological sample, said reagent comprising at least one of a capture probe and a detection probe, wherein said capture probe and said  
15 detection probe each comprise a nucleotide sequence that is hybridizable to at least a segment of a nucleic acid according to claim 1, and wherein said capture probe and said detection probe, if they are both present, have nucleotide sequences that are at least partially  
20 different from one another.

12. The reagent according to claim 11, wherein said capture probe is attached to a solid support.

13. The reagent according to claim 12, wherein said capture probe is directly attached to said solid support.

25 14. The reagent according to claim 12, wherein said capture probe is indirectly attached to said solid support.

15. The reagent according to claim 11, wherein said detection probe is labelled by a marker selected from the

group consisting of radioactive isotopes, enzymes capable of hydrolyzing a chromogenic, fluorogenic or luminescent substrate, chromophoric chemical compounds, fluorogenic compounds, luminescent compounds, nucleotide base analogs, and biotin.

5       16. The reagent according to claim 15, wherein said enzymes are selected from the group consisting of peroxidase and alkaline phosphatase.

10      17. The reagent according to claim 11, comprising at least one primer comprising a nucleotide sequence that allows hybridization to at least a segment of a nucleic acid which comprises a nucleotide sequence that is identical, complementary, antisense or equivalent to a first sequence starting at nucleotide 1232 and ending at 15     nucleotide 1825 of SEQ ID NO:1.

18. A method for detection and/or identification of Trypanosoma cruzi in a biological sample, comprising exposing to at least one probe according to claim 5 denatured DNA extracted from Trypanosoma cruzi or DNA 20     obtained by reverse transcription of RNA extracted from Trypanosoma cruzi; and detecting hybridization of said probe.

25      19. A method for detection and/or identification of Trypanosoma cruzi in a biological sample, comprising exposing extracted RNA from Trypanosoma cruzi to at least one probe according to claim 5; hybridizing said probe with said RNA; and detecting said hybridization.

20. The method according to claim 18, wherein before said DNA is exposed to said probe, said DNA is

amplified in the presence of an enzymatic system with at least one primer, wherein said primer comprises a nucleotide sequence that is hybridizable to a nucleic acid sequence that is identical, complementary, antisense 5 or equivalent to a sequence identified in SEQ ID NO:1.

ABSTRACT OF THE DISCLOSURE

The nucleotide sequence of Tc100, a gene encoding PTc100, a new *Trypanosoma* antigen, and the amino acid sequence of PTc100 are described. Tc100 and PTc100, or 5 fragments thereof, modified or otherwise, can be used directly or indirectly for the detection of *Trypanosoma cruzi*, or for the monitoring of the infection generated by *Trypanosoma cruzi*, in man or in animals.

36-42280-5522ET60

1/2

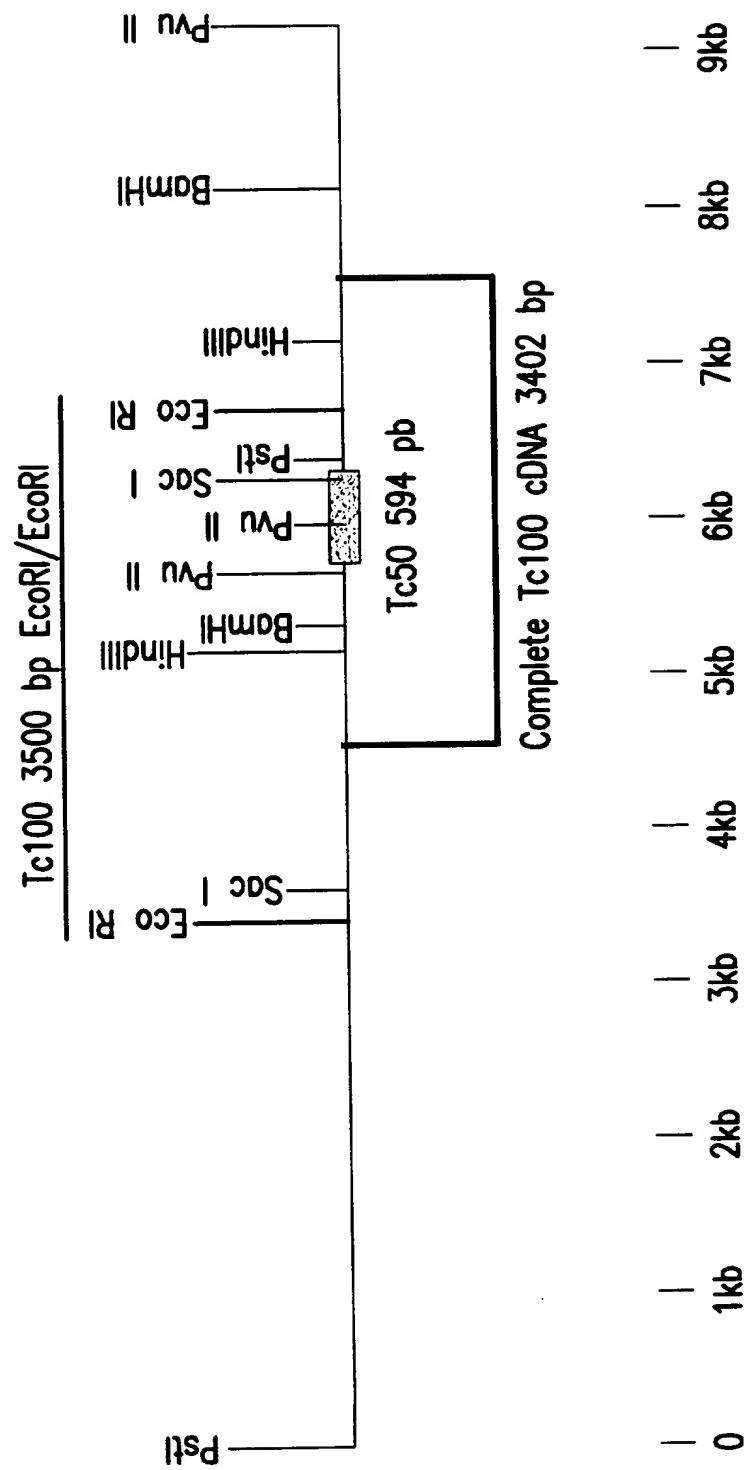


FIG. 1

2/2

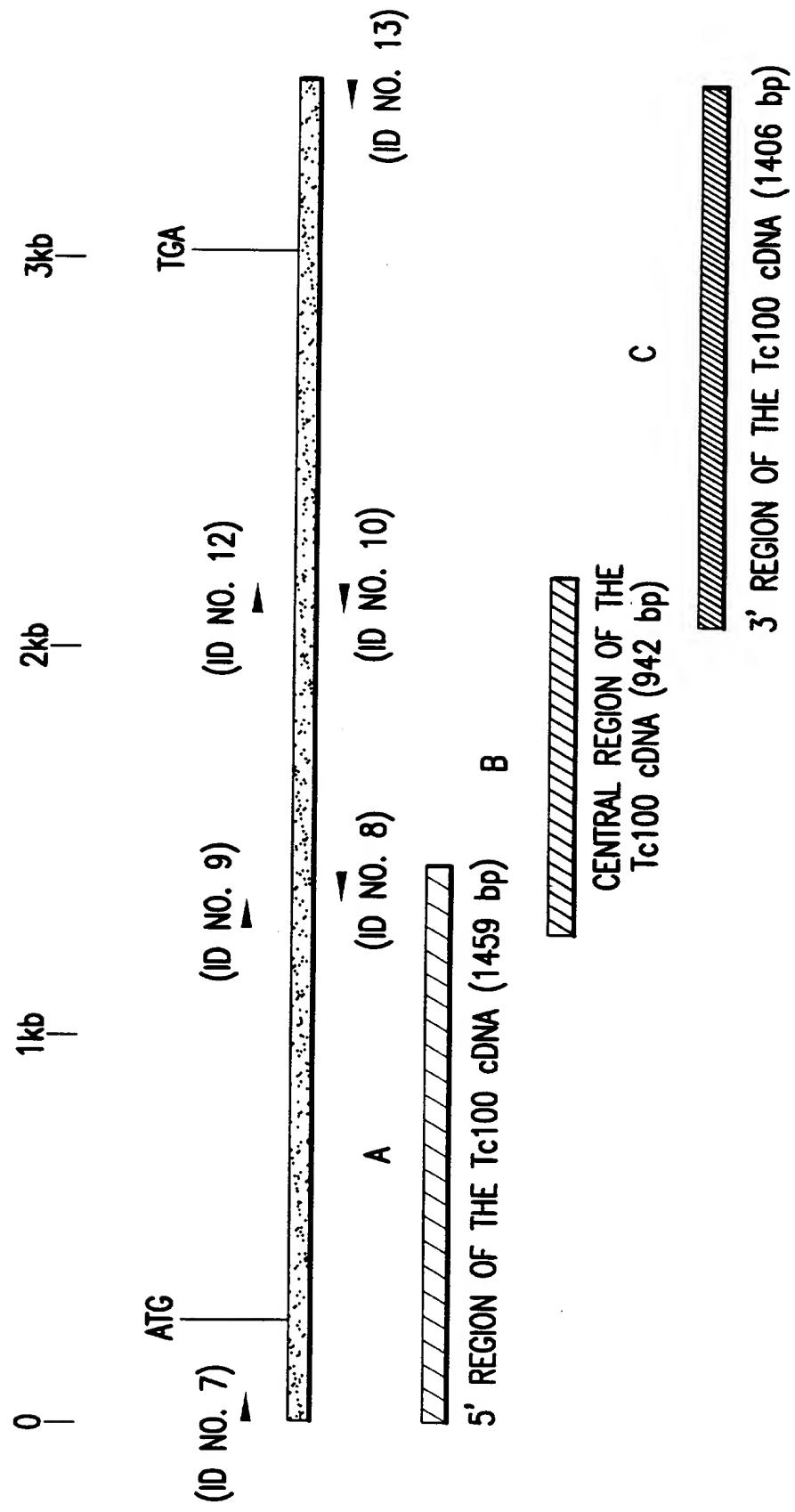


FIG.2

# APPLICATION FOR UNITED STATES PATENT DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name; that

I verily believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:  
**NEW TRYPANOSOMA CRUZI ANTIGEN, GENE ENCODING THEREFOR AND METHODS OF DETECTING AND TREATING CHAGAS DISEASE**

described and claimed in the specification:

**Check one**

- \*a.  attached hereto.
- b.  filed on June 7, 1995 as Application No. 08/480,917 and amended on \_\_\_\_\_ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

Under Title 35, U.S. Code §119, the priority benefits of the following foreign application(s) and/or United States provisional application(s) filed within one year prior to this application are hereby claimed:

French Patent Application No. 94 10132, filed August 12, 1994

The following application(s) for patent or inventor's certificate on this invention were filed in countries foreign to the United States of America either (a) more than one year prior to this application, or (b) before the filing date of the above-named foreign priority application(s) and/or United States provisional application(s):

I hereby appoint the following as my attorneys of record with full power of substitution and revocation to prosecute this application and to transact all business in the Patent Office:

James A. Oliff, Reg. No. 27,075; William P. Berridge, Reg. No. 30,024;  
 Kirk M. Hudson, Reg. No. 27,562; Thomas J. Pardini, Reg. No. 30,411;  
 Edward P. Walker, Reg. No. 31,450; Robert A. Miller, Reg. No. 32,771 and  
 Mario A. Costantino, Reg. No. 33,565.

**ALL CORRESPONDENCE IN CONNECTION WITH THIS APPLICATION SHOULD BE SENT TO OLIFF & BERRIDGE,  
 PLC, P.O. BOX 19928, ALEXANDRIA, VIRGINIA 22320, TELEPHONE (703) 836-6400.**

I hereby declare that I have reviewed and understand the contents of this Declaration, and that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1	<b>Typewritten Full Name of First or Sole Inventor</b>			Given Name	Middle Initial	Family Name
				Glaucia	S	PARANHOS-BACCALA
2	<b>**Inventor's Signature:</b>			<i>Glaucia Paranhos Baccala</i>		1995
3	<b>**Date of Signature:</b>			8	Day	Year
	Residence:	Lyon		Month	FRANCE	Country
	Citizenship:	Brazil and Switzerland		State or Province		
	Post Office Address:	75 cours Gambetta				
	(Insert complete mailing address, including country)	69003 Lyon, FRANCE				

\*If Box (a.) is checked, this form may be executed only when attached to the specification (including claims).

\*\*Note to Inventor: Please sign name exactly as it appears above and insert actual date of signing.

**IF THERE IS MORE THAN ONE INVENTOR USE PAGE 2 AND PLACE AN "X" HERE**

(Discard this page in a sole inventor application)

1	Typewritten Full Name of Joint Inventor	Given Name	Middle Initial	Family Name
2	Inventor's Signature	<u>LESENECHAL MYLENE</u>		
3	Date of Signature	<u>06/23/1995</u>		
Residence	Villeurbanne City	State or Province	FRANCE Country	
Citizenship	France			
Post Office Address (Insert complete mailing address, including country)	84 rue Anatole France 69100 Villeurbanne, FRANCE			
1	Typewritten Full Name of Joint Inventor	Given Name	Middle Initial	Family Name
2	Inventor's Signature	<u>JOLIVET Michel</u>		
3	Date of Signature	<u>06/27/95</u>		
Residence	Bron City	State or Province	FRANCE Country	
Citizenship	France			
Post Office Address (Insert complete mailing address, including country)	16 avenue des Colonnes 69500 Bron, FRANCE			
Typewritten Full Name of Joint Inventor	Given Name	Middle Initial	Family Name	
Inventor's Signature				
Date of Signature				
Residence	City	State or Province	Country	
Citizenship				
Post Office Address (Insert complete mailing address, including country)				
Typewritten Full Name of Joint Inventor	Given Name	Middle Initial	Family Name	
Inventor's Signature				
Date of Signature				
Residence	City	State or Province	Country	
Citizenship				
Post Office Address (Insert complete mailing address, including country)				
1	Typewritten Full Name of Joint Inventor	Given Name	Middle Initial	Family Name
2	Inventor's Signature			
3	Date of Signature			
Residence	City	State or Province	Country	
Citizenship				
Post Office Address (Insert complete mailing address, including country)				

Note to Inventor: Please sign name on line 2 exactly as it appears in line 1 and insert the actual date of signing on line 3.

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